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Howell's Gumweed (*Grindelia howellii*) Genetic Diversity and Conservation

Lab Report

NFGEL Project #333

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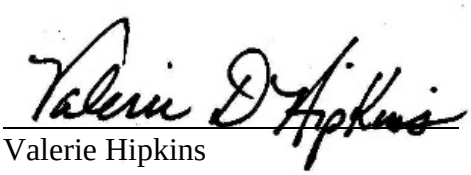

Valerie Hipkins
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SUMMARY

We used genetic data from nine populations of *Grindelia howellii*, one population of *Grindelia squarrosa*, and one putative hybrid population to examine genetic diversity and variability. The general results are provided in the table below:

Analysis	Question	Results
Data quality	Are the markers appropriate?	Data quality is good - no evidence of missing genetic data or correlations between loci
Inbreeding	Are populations inbred and in poor genetic health?	Measures of inbreeding are not particularly high; SO-148 has the highest measure of inbreeding.
Genetic diversity	Is there high genetic diversity in the populations?	Genetic diversity is not low in the *G. howellii* populations, but we don't have enough *G. squarrosa* populations to make a determination in that species.
Genetic variability	Are populations genetically similar to each other?	The combined populations, including both species, are distinct from each other. *G. howellii* populations from the Seely Lake area (Montana populations) are very similar to each other. Most of the genetic variability is within individuals, not among populations or individuals
Genetic versus geographic distance	Are geographically distant populations genetically distant as well?	There is a correlation between genetic and geographic distance across all study populations. Within *G. howellii* Montana populations, there is no correlation.
Hybrids	Are *G. howellii* and *G. squarrosa* hybridizing?	We don't have enough samples of *G. squarrosa* and putative hybrids to answer this question.

We used the data to answer the following questions.

(1) Are the *G. howellii* populations' sizes, genetic diversity, and variability ideal to preserve genetic integrity?

The number of total individuals in the sampled populations varies greatly (30 – 8,800), but genetic diversity is not strikingly different across populations. If we accept the general northern, central, and eastern groupings of the Seely Lake (Montana) populations as 'meta-populations', the northern meta-population has lower genetic diversity than the central and eastern meta-populations. If the northern populations are large, the relatively low genetic diversity could be of concern.

(2) How do the genetics of *G. howellii* interact with conservation efforts, restoration, and management for this species?

Our results do not indicate any immediate threat to the sampled populations in terms of low genetic diversity, and indeed the Montana populations seem to be acting as a large meta-

population, with few genetic differences between populations. In particular, SO-143 is found along a road that may be developed in the future. Looking at its genetic diversity and the STRUCTURE results, it is not distinctly different from SO-148 and SO-82/83. However, it does have a private allele found only in that population. If the population is destroyed, it may be worth transplanting individuals and/or collecting seeds to add to the SO-148 and SO-82/83 populations.

(3) How closely related are *G. howellii* and *G. squarrosa*?

Species identification within *Grindelia* is hard due to the wide range of morphological variation within and among species, and previous genetic studies have found that 'species' aren't always unique genetic units. The putative hybrid population in this study, SO-171, is quite distinct from the other *G. howellii* Montana populations. However, if it is a hybrid, we'd expect it to share genetics with both the Blue Mountain population of *G. squarrosa* and Montana populations of *G. howellii*. However, Blue Mountain, though different from the Montana populations, isn't strikingly so – as a different species, we'd expect it to be quite distinct. The SO-171 population could be a hybrid, or it could be that geographic distance from the other Montana populations has led to genetic differentiation. The Clear Creek population is also quite distinct. This could be because of geographic distance or because it is not *G. howellii*. At this point, we recommend collecting from more populations of *G. squarrosa* and putative hybrid populations.

(4) What is the relationship between geographic range size and levels of genetic diversity in lineages?

Without more *G. squarrosa* populations, we can't relate population genetic levels to range sizes. The single included putative *G. squarrosa* population doesn't have markedly different levels of genetic diversity compared to the *G. howellii* populations.

INTRODUCTION

Grindelia howellii (Asteraceae; Howell's Gumweed) is a restricted species endemic to Montana and Idaho (USDA National Resources Conservation Service, 2017). There is debate over classification of the species; *G. howellii* has been grouped with *Grindelia paysonorum* (Bartoli and Tortosa, 2012) and considered similar to *Grindelia nana* (Strother and Wetter, 2006). However, as Strother and Wetter (2006) note, *G. howellii* has distinctive glands on the stem and leaves, making it sticky to the touch. The most recent molecular phylogeny of the genus confirms its place in the "Pacific" Clade with other species in the region (Moore et al., 2012). However, this genetic study only tested one herbarium specimen (accession) for *G. howellii*, and other species with multiple accessions per species were polyphyletic. These results point to a complicated relationship between taxonomy and evolutionary differences in the genus.

Beyond these taxonomic issues, *G. howellii* is a considered a sensitive species in Montana by the US Forest Service. It is found in open habitats such as roadsides and open wetlands (Lesica, Lavin, and Stickney, 2012), and could be negatively impacted by road management or development. Also, given its restricted range, populations may have low genetic diversity and/or require protection in order to perpetuate the species. Despite local abundance (Lesica, Yurkewycz, and Crone, 2006), restricted endemics can be limited by dispersal distances and low genetic diversity. Their limited range and habitat preference can make them more susceptible to fragmentation and loss of genetic diversity (Wagenius, Lonsdorf, and Neuhauser, 2007). However, endemism and restricted range does not always indicate low genetic diversity or high inbreeding (Williams et al., 2016). In addition to occupying a small range, *G. howellii* may hybridize with other species in the genus, such as *Grindelia squarrosa* (Lesica, Lavin, and Stickney, 2012), which is widely distributed across the United States. *Grindelia squarrosa* has reportedly hybridized with at least one other species, *G. nana* (Moore et al., 2012). Hybrid populations may exist where species overlap in their ranges, and produce morphologically intermediate individuals.

Here, we used microsatellite loci and samples from *G. howellii* and *G. squarrosa* populations to examine genetic diversity and possible hybridization. The main questions to be answered in this study were: 1) Are the *G. howellii* populations' sizes, genetic diversity, and variability ideal to preserve genetic integrity? 2) How do the genetics of *G. howellii* interact with conservation efforts, restoration, and management for this species? 3) How closely related are *G. howellii* and *G. squarrosa*? 4) What is the relationship between geographic range size and levels of genetic diversity in *G. howellii* and *G. squarrosa*?

MATERIALS AND METHODS

Sampling

Leaf samples were collected from nine populations of *G. howellii*, one population of *G. squarrosa,* and one population of a putative *G. squarrosa* X *G. howellii* hybrid[1] (Fig. 1; Table 1). The Clear Creek population of *G. howellii* occurs in Idaho while the remaining populations occur in Montana. At each population, 3-5 leaves per individual were collected from 16 to 33 individual plants; thus, a total of 280 plants were sampled. The only exception to this occurred at the *G. squarrosa* "Blue Mountain" population, where 3-5 leaves were collected from 7 individual plants. We ran analyses on three different datasets: all samples from all 11 populations, just *G. howellii* (excluding *G. squarrosa* and the putative hybrid population), and just the Montana populations of *G. howellii* (which excluded the Clear Creek, Idaho population of *G. howellii).*

Microsatellite Loci

DNA was extracted from fresh leaf tissue using a DNEasy 96 Plant Kit (Qiagen Valencia, California, USA) and quantified using a ThermoFisher Quant-iT PicoGreen dsDNA Assay Kit visualized on a Molecular Devices Gemini XPS plate fluorometer. The DNA samples were genotyped using 6 microsatellite loci (Table 2). Loci were originally developed for *Grindelia* species (Abercrombie et al., 2009), and have been used successfully in other studies of *Grindelia* (Moore, Moore, and Baldwin, 2014).

The loci were amplified with 10 µl PCR reactions (1.5U HotStar Taq, 0.2 mg/ml BSA, 0.4 µM forward primer, 0.4 µM reverse primer, 1 µl of 10x buffer with 15 mM $MgCl_2$, 125 µM dNTPs, 10 ng DNA; 95°C for 5 min, [94°C for 30 sec, 55°C for 45 sec (-0.5°C per cycle), 72°C for 45 sec, repeat 20x] [94°C for 30 sec, 45°C for 45 sec, 72°C for 45 sec, repeat 10x], 72°C for 10 min). The samples were run on an ABI3130xl with ROX as an internal standard, with the GRIN026 amplification product diluted 1:30. The loci were scored by hand using GeneMarker v1.95 (SoftGenetics LLC, 2001-2010).

Analyses

We checked for null alleles using Micro-Checker with 1000 replicates (Van Oosterhout et al., 2004) and examined linkage disequilibrium (LD) among loci using Genepop (Raymond and Rousset, 1995; Rousset, 2008) with the following Markov chain parameters: 100 batches, 1000 iterations per batch, and dememorisation of 1000. We also used Genepop to determine F_{IS} values and their significance with default options.

We used GenAlEx v6.503 (Peakall and Smouse, 2006; Peakall and Smouse, 2012) to calculate diversity statistics (number of alleles, effective number of alleles, observed and expected heterozygosity, private alleles, F_{IS}, F_{ST}, AMOVA), visualize Nei's genetic distance

[1] The Montana Natural Heritage Program botanist proposed the idea that this population could be a hybrid because morphologically plants exhibit leaves that barely clasp the stem and have a lower density of stipitate-glandular hairs on the stem making them less sticky.

(Nei, 1972) using Principal Coordinates Analysis (PCoA) ordination, and run Mantel tests of geographic and Nei's genetic distance with 999 permutations.

We used the program STRUCTURE (Pritchard, Stephens, and Donnelly, 2000) to look for patterns of genetic structure. We used an admixture model with 1,000,000 generations with 100,000 generations burn-in, with 5 iterations of K from one to the number of sampled populations – 1, which depended on the dataset. We used the method described in Evanno et al. (2005) as implemented in STRUCTURE HARVESTER (Earl and vonHoldt, 2012) to determine the optimal value of K, or number of genetic clusters in the data.

RESULTS

Locus Amplification

Two loci used, GRIN24 and GRIN113, had evidence for null loci. For a large number of individuals (99 of 280 plants for GRIN24, and 20 of 280 plants for GRIN113), these loci did not amplify across multiple replicate PCR and ABI runs. For null loci, the entire locus fails to amplify, which leads to missing data. Null loci can result from a mutation in the primer sequence, which can be confirmed by sequencing the locus and primer region. To deal with the missing data in this study, we inferred loci frequencies when possible in GenAlEx.

Locus GRIN68 was monomorphic for all individual plants except for one individual in population SO-171 from the putative hybrid population. This means that analyses that include only *G. howellii* populations effectively used five loci instead of six.

Data Quality

We also tested for null alleles, which are different from null loci. In the case of null alleles, a particular allele fails to amplify, which can lead to potentially high estimates of homozygosity. MicroChecker found no evidence for null alleles in this dataset. Genepop also found no evidence for linkage disequilibrium ($p > 0.08$); however, the relationship between GRIN68 and GRIN113, GRIN24, and GRIN45 could not be evaluated. This is likely because of the lack of variation in GRIN68.

Population Genetic Diversity

A combination of different measures can be used to evaluate population genetic diversity. Populations with high genetic diversity generally have low inbreeding coefficients, a high number of effective alleles, and similar measures of observed and expected heterozygosity. For all populations of *Grindelia* in this study, the inbreeding coefficient F_{IS} (low values indicate less inbreeding) is quite variable (Table 3), with possible inbreeding at SO-148. The overall estimate of F_{IS} across populations is 0.211. The number of alleles (N_a) is similar across populations, which in turn is similar to the effective number of alleles (N_e). There is a similar pattern with observed heterozygosity (H_O) and expected heterozygosity (H_e), with slightly lower values of observed versus expected heterozygosity. Although individual loci within populations were significantly different from Hardy-Weinberg equilibrium, no population had all loci significantly different, and no locus was consistently significantly different across all populations. However,

GRIN113 was often significantly different. Sampling additional populations would allow us to evaluate if values of genetic diversity in *G. howellii* populations are significantly different from *G. squarrosa*. However, this analysis is not possible given that only one *G. squarrosa* population was sampled.

One measure of population differentiation is F_{ST}, which measures population differentiation and ranges from zero to one, with higher values indicating high population differentiation (Table 4). For all populations, $F_{ST} = 0.37$ which indicates some differentiation. For the *G. howellii* populations, $F_{ST} = 0.28$, which may reflect the genetic difference between Clear Creek and the SO populations. For the Montana populations, $F_{ST} = 0.13$, which indicates little differentiation across the SO populations. These F_{ST} measures mirror the results of the AMOVA tests. For all datasets, most variation was found within individuals. However, the Montana *G. howellii* populations had the least amount of variation among populations (13%) compared to all *G. howellii* populations (28%) and all populations (37%). In addition, private alleles (alleles found in only one population) can be an indication of population differentiation (Table 2). The Clear Creek population had the most private alleles, with 7, followed by the Blue Mountain population with 4.

Population Structure

The PCoA ordination (Fig. 2a) shows that the putative hybrid population SO-171, Clear Creek, and Blue Mountain populations are genetically different from the Montana *G. howellii* populations, and seems to correspond with geographic distance. Looking across the Montana *G. howellii* populations (Fig. 2b) there is loose cluster of the northern populations (SO-1, SO-172, and SO-52) and eastern populations (SO-11, SO-82/83, SO-143, and SO-148) with SO-115 located in a somewhat different position. This is supported by the Mantel test results. Across all populations, there was a significant correlation between geographic and genetic distance ($p = 0.04$, $R^2 = 0.9$). A significant correlation between geographic and genetic distance is also found across all *G. howellii* populations ($p = 0.04$, $R^2 = 0.43$). However, no significant correlation between geographic and genetic distance is found for the Montana *G. howellii* populations alone ($p = 0.4$, $R^2 = 0.0004$).

The STRUCTURE and STRUCTURE Harvester results show clear differences between SO-171, Clear Creek, and the rest of the populations, with $K = 4$ as the optimal number of clusters (Fig. 3). For all *G. howellii*, the most likely number of clusters is $K = 2$, with clear separation between Clear Creek and the SO populations. For just the Montana *G. howellii* populations, the most likely number of clusters is $K = 3$, which shows the distinction between northern populations (S0-52, SO-4, and SO-1), eastern populations (SO-82/83, SO-143, and SO-148), and the two central populations (SO-11 and SO-115).

DISCUSSION

In general, we found little evidence of low genetic diversity in populations of *Grindelia howellii.* Without more samples of *Grindelia squarrosa*, however, we are unable to compare

genetic diversity of *G. howellii* to a more wide-spread species or definitively identify a putative hybrid population. We discuss the specific project questions below.

(1) G. howellii populations' genetic integrity and variability

Our data shows that the Clear Creek population of *G. howellii* in Idaho is distinct from the SO populations from Montana. It has a large number of private alleles, and consistently separates out from the other populations in the PCoA ordination and the STRUCTURE analysis. We suggest collecting more populations from Idaho and/or verifying the identification of the Clear Creek, Idaho population using Flora of North America and Bartoli and Tortosa (2012) botanical keys.

Concentrating on the Montana *G. howellii* populations, our measures of diversity including number of alleles, number of effective alleles, and observed and expected heterozygosity are not particularly low. In terms of variability and population structure, we found that geographic distance between the Montana populations was not correlated with genetic distance, and F_{ST} is low, indicating that these populations are genetically similar and likely exchanging genes though pollination or seed dispersal. We do see indications of some differences between populations using STUCTURE, with three general 'meta-populations'. The northern meta-population has relatively lower genetic diversity that the central and eastern meta-populations. If the northern populations are large in terms of absolute number of individuals, the relatively low genetic diversity could be of concern.

Inbreeding doesn't appear to be a concern in most of the sampled populations. The exception is population SO-148, which has the highest measure of inbreeding and a low observed heterozygosity. Inbreeding may be low overall in *G. howellii* due to self-incompatibility which is widespread in the family Asteraceae (Charlesworth, 1985). Although a barrier to inbreeding, if self-incompatibility is present in the genus or species it could limit the number of potential mates in small populations.

(2) G. howellii genetics as it relates to conservation efforts, restoration, and management

The historic habitat of *G. howellii* is likely shallow wetlands and moist meadows (Lesica, Lavin, and Stickney, 2012), but it also currently favors disturbed habitats such as roadsides. Our results do not indicate any immediate threat to the sampled populations in terms of low genetic diversity, and indeed the Montana *G. howellii* populations seem to be acting as a large meta-population, with few genetic differences between populations. The sampled populations are found within a larger matrix of populations in the Seely Lake and Ovando valleys.

In particular, SO-143 is found along a road that is proposed for re-construction. Looking at its genetic diversity and the STRUCTURE results, it is not distinctly different from SO-148 and SO-82/83. However, it does have a private allele found in no other populations. It is a large population (estimated 8,800 individuals) which may harbor additional genetic diversity. If the population is to be destroyed, it may be worth transplanting individuals and/or collecting seeds to add to the SO-148 and SO-82/83 populations.

(3) Relationship between G. howellii and G. squarrosa

Species identification within *Grindelia* is difficult due to the wide range of morphological variation within and among species, and previous genetic studies have found that 'species' aren't always unique genetic units (Moore et al., 2012; Moore, Moore, and Baldwin, 2014). Moore et al. (2012) found that individuals from the same *Grindelia* 'species' did not group together using a phylogenetic approach. Previous work by Moore, Moore, and Baldwin (2014) using the same microsatellites in California populations found that populations segregated by ecotype, not ploidy level. In general, however, Moore et al. (2012) found that *G. howellii* and *G. squarrosa* were in different clades (genetic groups), with *G. howellii* in the Pacific Clade and *G. squarrosa* in a widespread Eastern United States Clade.

However, there could be hybridization between the two species. The main barrier to determining the relationship between *G. howellii* and *G. squarrosa* and the existence of hybrid populations is that only one population of *G. squarrosa* and one population of a putative hybrid were included in this genetic study. The putative hybrid population in this study, SO-171, is quite distinct from the other Montana populations. However, if it is a hybrid, we'd expect it to share genetics with both the Blue Mountain population of *G. squarrosa* and the Montana *G. howellii* populations. However, Blue Mountain, though different from the Montana populations, isn't strikingly so, and as a different species, we'd expect it to be quite distinct. The SO-171 population could be a hybrid, or it could be that geographic distance from the other Montana populations has led to genetic differentiation. Also, the identification of *G. squarrosa* from this population is in doubt as both *G. squarrosa* and *G. nana* occur in the county (A. Pipp, *pers. comm.*). Future field work will confirm the identification of this population.

Similarly, the Clear Creek population is also quite distinct. This could be because of geographic distance or because it is not *G. howellii*. We recommend collecting from more populations of *G. squarrosa* and putative hybrid populations. However, the SO-populations excluding SO-171 appear to be a cohesive genetic group with morphology consistent with *G. howellii*.

(4) Geographic range size and levels of genetic diversity

Without more *G. squarrosa* populations sampled, we can't relate population genetic levels to range sizes. The single included putative *G. squarrosa* population doesn't have markedly different levels of genetic diversity compared to *G. howellii* populations. Future collections should be made of specimens with clear *G. squarrosa* characters and of specimens with clear *G. nana* characters, especially given that these species may hybridize in parts of Montana.

CONCLUSIONS

Grindelia howellii populations are not genetically depauperate compared to a single sampled population of a wide-spread congener species, *G. squarrosa*. The most genetically distinct *G. howellii* population in the study is from Clear Creek. The putative hybrid population SO-171 and the Blue Mountain (*G. squarrosa*) are also genetically distinct. The Montana *G.*

howellii can be divided into three meta-populations, north, central, and east, but appear to exchange alleles. There is currently no evidence for hybridization, but additional populations of *G. squarrosa, G. nana,* and putative hybrids are needed to fully test that hypothesis.

Table 1 – Eleven *Grindelia* populations were sampled for genetic analysis.

Species	Population Name[1]	Latitude, Longitude	Estimated population area (acres)	Estimated # of individuals	# of samples
G. howellii	SO-1	47.438060, -113.645650	4	>300	23
G. howellii	SO-11	47.153300, -113.461790	13	>1340	31
G. howellii	SO-52	47.469470, -113.632820	<1	30	16
G. howellii	SO-82/83	47.073917, -113.219754	3	>500	29
G. howellii	SO-115	47.092840, -113.420140	<0.5	89	33
G. howellii	SO-143	47.110151, -113.184390	61	8800	29
G. howellii	SO-148	47.115588, -113.218901	47	1530	29
G. howellii	SO-172[2]	47.437780, -113.657373	<2	>100	26
G. howellii x squarrosa	SO-171	46.502610, -112.531100	3	>300	27
G. howellii	Clear Creek, Idaho	46.080200, -115.694900	NA	NA	30
G. squarrosa	Blue Mountain[3]	46.825800, -114.099500	NA	NA	7

1 - All populations are in Montana unless noted
2 - Originally labelled incorrectly in the field as coming from SO-4. It was collected from SO-172.
3 - Averaged location for three sub-locations: (46.8288, -114.1), (46.8237, -114.102), (46.8249, -114.09)

Table 2 – Microsatellite primers used in this study.

Locus	F Primer	R Primer	Forward primer dye	Size Range
GRIN024	TCGACTCGAATTCTCATGTGTC	TACATCGCCCAAATCAATCA	NED	218-238
GRIN026	CCATAGGCGGTATACAATAGCC	CCCAATCATCCAACGAAATC	FAM	211-246
GRIN035	GCTTCAAACTCAAGCAAGCA	CACAAAAGCATGTGCATCAA	HEX	214-226
GRIN045	TTGACTATCATTTGAACCATCCA	AGAGAAACATCCGTGCGTGT	NED	399-419
GRIN068	TGGGCTGACTCCTGCTTAAT	TGCAGATTCCGAAAACACAA	HEX	352-354
GRIN113	CGTGGTCTAAACGGGGTATC	CCATTCCTCATCGAACTCTTG	FAM	454-481

Note: All loci from Abercrombie et al. 2009, see text for annealing temperatures

Table 3 – Diversity statistics for eleven populations of *Grindelia*.

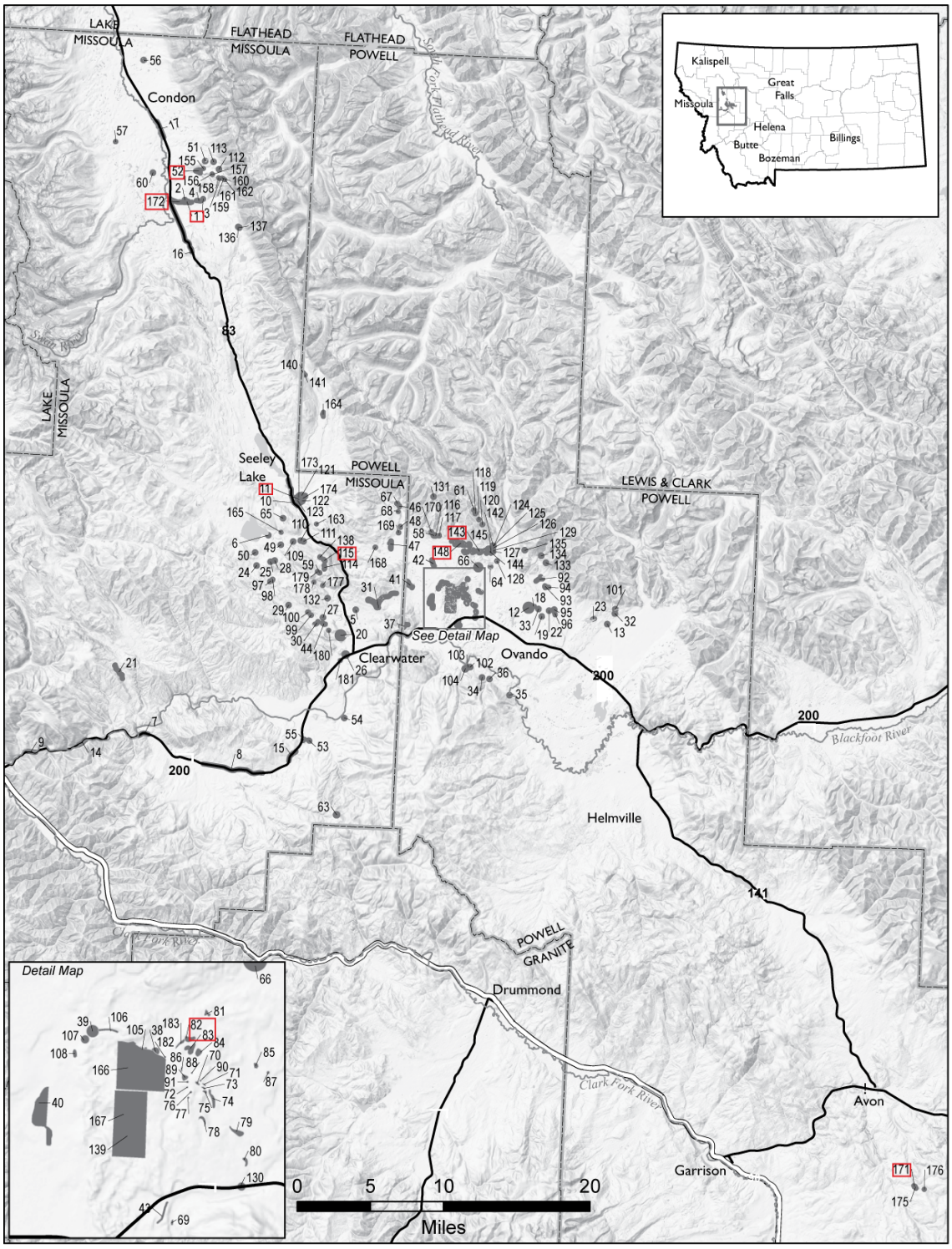
Species	Population Name	F_{IS}	Na	Ne	Ho	He	Hardy-Weinberg Equilibrium	Private alleles - all populations	Private alleles - *G. howellii*	Private alleles - Montana populations of *G. howellii*	Notes
G. howellii	SO-1	0.297	1.83	1.49	0.15	0.21	Locus 113 significantly different	1	1	1	3 of 6 loci are monomorphic
G. howellii	SO-11	0.219	3.17	1.75	0.23	0.30	Loci 24 and 113 significantly different	2	2	2	1 of 6 loci are monomorphic
G. howellii	SO-52	-0.146	1.50	1.26	0.17	0.14	Not significant	0	0	0	4 of 6 loci are monomorphic
G. howellii	SO-82/83	0.163	2.67	1.7	0.26	0.31	Loci 24 and 113 significantly different	1	1	1	1 of 6 loci are monomorphic
G. howellii	SO-115	0.159	2.00	1.43	0.20	0.24	Not significantly different	0	0	0	2 of 6 loci are monomorphic
G. howellii	SO-143	0.240	2.33	1.61	0.19	0.24	Locus 113 significantly different	1	1	1	2 of 6 loci are monomorphic
G. howellii	SO-148	0.374	2.83	1.89	0.22	0.34	Locus 113 significantly different	0	0	1	1 of 6 loci are monomorphic
G. howellii	SO-172	0.161	1.67	1.55	0.21	0.25	Locus 113 significantly different	0	0	0	3 of 6 loci are monomorphic
G. howellii x squarrosa	SO-171	0.293	1.83	1.42	0.16	0.24	Loci 24 and 68 significantly different	1	NA	NA	1 of 6 loci are monomorphic
G. howellii	Clear Creek Idaho	0.205	2.83	1.58	0.26	0.32	Loci 26 and 45 significantly different	7	7	NA	1 of 6 loci are monomorphic
G. squarrosa	Blue Mountain[2]	0.125	2.83	1.49	0.27	0.29	Not significantly different	4	NA	NA	1 of 6 loci are monomorphic

Note: Na = number of alleles, Ne = number of effective alleles, Ho = observed heterozygosity, He = expected heterozygosity

Table 4 – Measures of F_{ST} and AMOVA results.

Dataset	F_{ST}	AMOVA results	Within individuals	Among populations	Among individuals
All populations	0.37	p<0.01	50%	37%	13%
G. howellii	0.28	p<0.01	57%	28%	15%
Montana *G. howellii*	0.13	p<0.01	69%	13%	18%

FIGURES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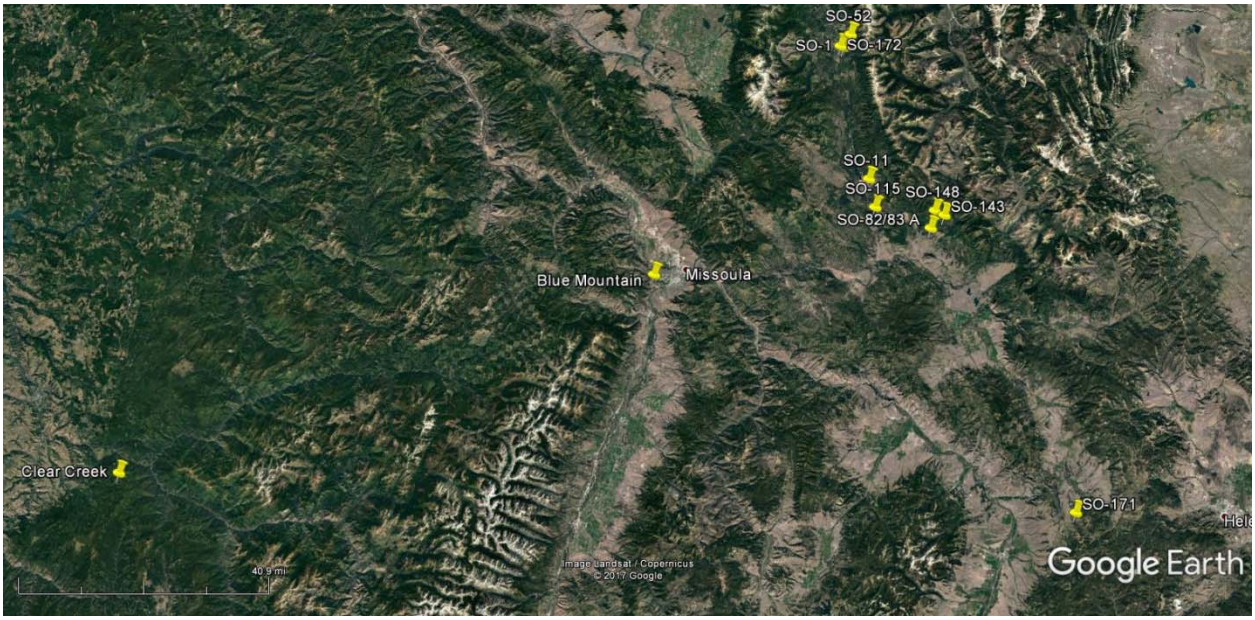
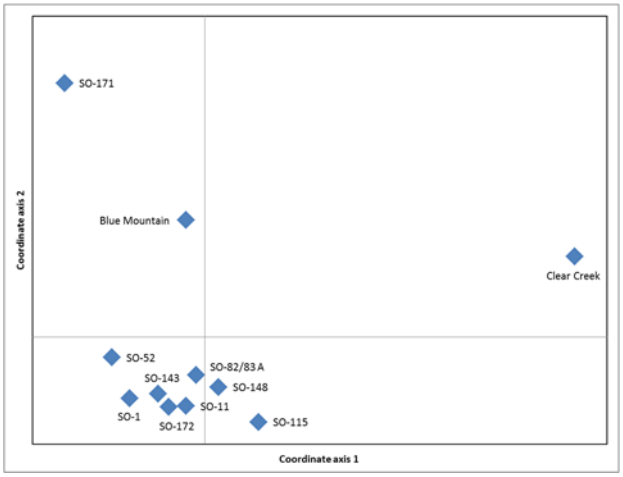
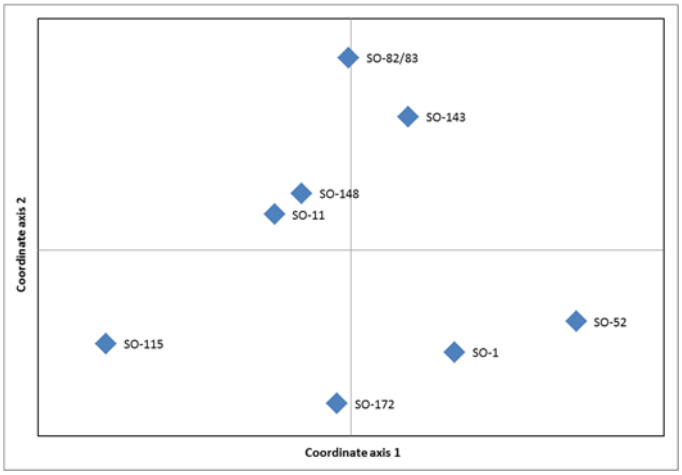


(b)

Figure 1 – (a) Known *Grindelia howellii* populations in the Seely Lake Area (SO populations). Sampled populations have red squares surrounding the population number. SO-171 is a putative hybrid population between *G. howellii* and *G. squarrosa*. Map created by Jamul Hahn. (b) All populations used in this study, including Blue Mountain (*Grindelia squarrosa*) and Clear Creek, ID (*G. howellii*). Map from Google Earth.





a. b.

Figure 2 – Principal Coordinate Analysis ordination of Nei's genetic distance for (a) All populations (first two axes explains 65.95% of the variation) and (b) Montana *G. howellii* populations (first two axes explains 84.22% of the variation).

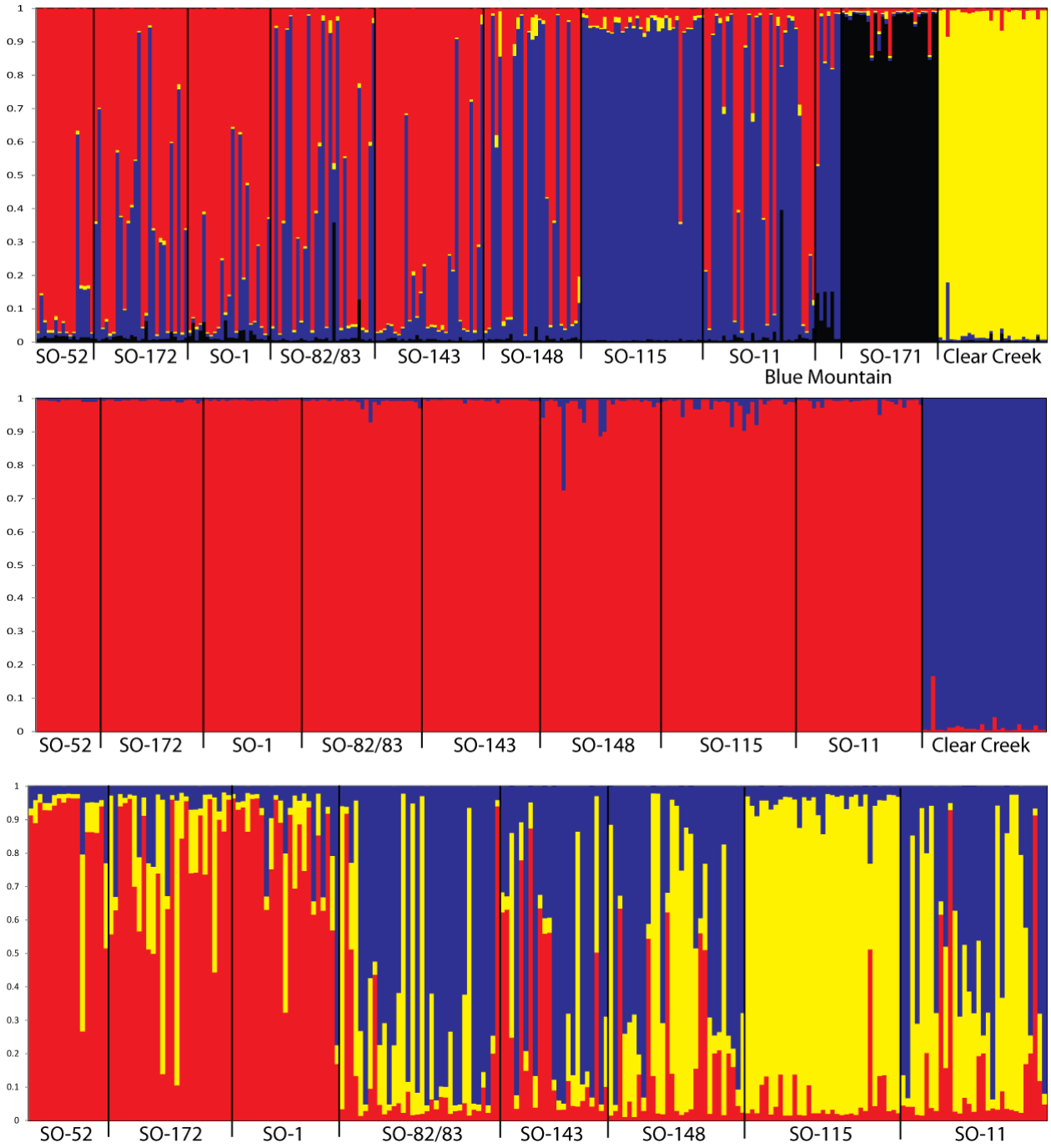


Figure 3 – STRUCTURE results for the three datasets. TOP: All 11 sampled populations including Blue Mountain (*Grindelia squarrosa*) and SO-171 (putative hybrid of *G. howellii* and *G. squarrosa*) (K=4). MIDDLE: *Grindelia howellii* populations, which excludes the putative hybrid SO-171 and Blue Mountain *G. squarrosa* populations (K=2). BOTTOM: Montana *G. howellii* populations (which excludes SO-171, Clear Creek, and *G. squarrosa* populations; K=3).

REFERENCES

ABERCROMBIE, L. G., C. M. ANDERSON, B. G. BALDWIN, I. C. BANG, R. BELDADE, G. BERNARDI, A. BOUBOU, et al. 2009. Permanent Genetic Resources added to Molecular Ecology Resources database 1 January 2009-30 April 2009. *Mol Ecol Resour* 9: 1375-1379.

BARTOLI, A., AND R. D. TORTOSA. 2012. Revision of the North American Species of Grindelia (Asteraceae). *Annals of the Missouri Botanical Garden* 98: 447-513.

CHARLESWORTH, D. 1985. Distribution of dioecy and self-incompatibility in angiosperms. *In* P. J. Greenwoog, P. H. Harvey, AND M. Slatkin [eds.], Evolution: essays in honor of John Maynard Smith, 237-268. Cambridge University Press, Cambridge, UK.

EARL, D. A., AND B. M. VONHOLDT. 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources* 4: 359-361.

EVANNO, G., S. REGNAUT, AND J. GOUDET. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology* 14: 2611-2620.

LESICA, P., R. YURKEWYCZ, AND E. E. CRONE. 2006. Rare plants are common where you find them. *American Journal of Botany* 93: 454-459.

LESICA, P., M. T. LAVIN, AND P. F. STICKNEY. 2012. Manual of Montana vascular plants. BRIT Press, Fort Worth.

MOORE, A. J., W. L. MOORE, AND B. G. BALDWIN. 2014. Genetic and Ecotypic Differentiation in a Californian Plant Polyploid Complex (Grindelia, Asteraceae). *PLoS One* 9: e95656.

MOORE, A. J., A. BARTOLI, R. D. TORTOSA, AND B. G. BALDWIN. 2012. Phylogeny, biogeography, and chromosome evolution of the amphitropical genus Grindelia (Asteraceae) inferred from nuclear ribosomal and chloroplast sequence data. *Taxon* 61: 211-230.

NEI, M. 1972. Genetic distance between populations. *American Naturalist* 106: 283-292.

PEAKALL, R., AND P. E. SMOUSE. 2006. GenAlEx 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes* 6: 288-295.

PEAKALL, R., AND P. SMOUSE. 2012. GenAlEx 6.5: Genetic analysis in Excel. Population genetic software for teaching and research - an update. *Bioinformatics* 10.1093/bioinformatics/bts460 10.1093/bioinformatics/bts460.

PRITCHARD, J. K., M. STEPHENS, AND P. DONNELLY. 2000. Inference of population structure using multilocus genotype data. *Genetics* 155: 945-959.

RAYMOND, M., AND F. ROUSSET. 1995. GENEPOP (Version 1.2): Population genetics software for exact tests and ecumenicism. *Journal of Heredity* 86: 248-249.

ROUSSET, F. 2008. genepop'007: a complete re-implementation of the genepop software for Windows and Linux. *Molecular Ecology Resources* 8: 103-106.

STROTHER, J. L., AND M. A. WETTER. 2006. Grindelia. *In* Flora of North America Editorial Committee [ed.], Flora of North America: Asteraceae. Oxford University Press.

USDA NATIONAL RESOURCES CONSERVATION SERVICE. 2017. PLANTS Database Website http://plants.usda.gov/ 2017.

VAN OOSTERHOUT, C., W. F. HUTCHINSON, D. P. M. WILLS, AND P. SHIPLEY. 2004. Micro-Checker: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes* 4: 535-538.

WAGENIUS, S., E. LONSDORF, AND C. NEUHAUSER. 2007. Patch aging and the S-allee effect: breeding system effects on the demographic response of plants to habitat fragmentation. *American Naturalist* 169: 383-397.

WILLIAMS, E. W., R. CHEUNG, C. SIEGEL, M. HOWARD, J. FANT, AND K. HAVENS. 2016. Persistence of the gypsophile Lepidospartum burgessii (Asteraceae) through clonal growth and limited gene flow. *Conservation Genetics* 17: 1201-1211.